VALIDATION OF RAPD MARKERS LINKED TO *Co-4* ANTHRACNOSE RESISTANCE ALLELES IN COMMON BEAN CULTIVAR PI 207.262

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The common bean breeding program which is being conducted at BIOAGRO/UFV, in Vicosa, MG, Brazil, uses the cultivar PI 207.262 as a source for anthracnose resistance and the "carioca-type" cultivar Rudá as the recurrent progenitor. Previous works have reported the presence of two independent dominant anthracnose resistance genes in PI 207.262, one of them being an allele of the Co-4 gene designated Co-4^3 (Alzate-Marin et al., 2000). Other alleles of this gene are present in cultivars TO (Co-4) and G 2333 (Co-4^2). RAPD markers OPY20 and OPJO1 are linked to the Co-4 gene in cultivar TO (Arruda et al., 2000) and markers OPH18 and OPAS13 are linked to the Co-4^2 allele in cultivars SEL 1308 and G2333 (Alzate-Marin et al., 2001; Young & Kelly, 1998). These markers are potential candidates to aid the selection of plants harboring the Co-4^3 allele derived from the cross between cultivars Rudá and PI 207.262. The main goals of the present work were: 1) to test markers OPY20, OPH18, OPJO1 and OPAS13 in two contrasting bulks of plants selected from an F_2 population from the cross Rudá vs PI 207.262 to identify the one showing the lowest number of recombinants in the susceptible bulk; 2) to use the selected marker to identify $F_{2:3}$ families with the Co-4^3 allele and 3) to determine the genetic distance between the selected marker and the Co-4^3 allele in $F_{2:3}$ families segregating for only one anthracnose resistance gene.

Population F_2 derived from crosses between Rudá (susceptible to most races races of Colletotrichum lindemuthianum) and the resistant cultivar PI 207.262 were used. Leaf DNA was extracted from the parents and from two bulks of F_2 plants contrasting for resistance to C. lindemuthianum pathotype 65, and amplified with primers flanking the markers OPY20, OPH18, OPJO1 and $OPAS13_{950C}$. Marker $OPAS13_{950C}$ was present in all resistant plants and absent in all susceptible plants (Figure 1). Ten $F_{2:3}$ families were obtained from individual F_2 plants harboring the $OPAS13_{950C}$ marker. These plants were inoculated with spores (1.2×10^6 spores/ml) from C. lindemuthianum pathotype 65 to select those segregating for only one anthracnose resistance gene.

The inoculation results showed that three out of the 10 $F_{2:3}$ families segregated for one gene only. DNA from 67 plants of these three families was tested positive for marker $OPAS13_{950C}$, confirming that this marker was indeed linked to the Co-4^3 allele. The genetic distance between the gene and the marker was 3.5 cM (Table 1). Our data demonstrated that the $OPAS13_{950C}$ marker can be used to follow alleles Co-4^2 and Co-4^3 in a breeding program. In our breeding program at BIOAGRO/UFV, this marker can be used to identify lines harboring the Co-4^3 in the cross Rudá vs PI 207.262 or to indirectly select for lines carrying the second gene present in cultivar PI 207.262.

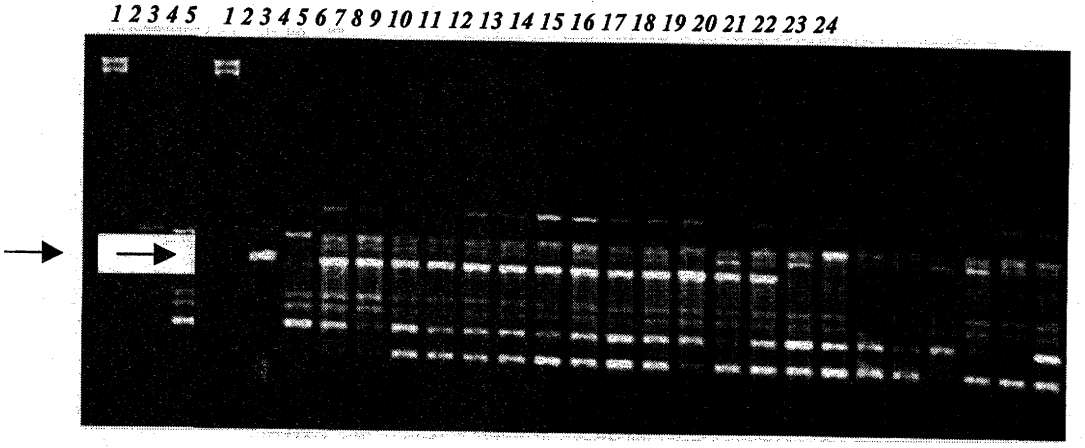

Figure 1. Electrophoretic analysis of amplification products obtained with primer OPAS13. Lanes are as follows: 1, lambda DNA digested with *Eco*RI, *Bam*HI and *Hin*dIII (size markers); 2, PI 207.262; 3, Rudá; 4- 16, F_2 plants resistant to *C. lindemuthianum* pathotype 65; 17-24 F_2 plants susceptible to to *C. lindemuthianum* pathotype 65. The arrow indicates marker $OPAS13_{950C}$ linked in coupling phase to the *Co-4*^{3} gene.

Table 1. Linkage analysis between molecular marker $OPAS13_{950C}$ and resistance allele *Co-4*^{3} in crosses involving cultivars Rudá and PI 207.262

Cross	*Locus tested*	*Expected ratio**	Observed ratio*	χ^2	*P*	*cM***
Rudá x PI 207.262	*Co-4*^{3}	*3:1*	*55:12*	*1.796*	*70.54*	-
Rudá x PI 207.262	*Co-4*^{3}/$OPAS13_{950C}$	*9:3:3:1*	*53:0:2:12*	*42.24*	*0.00*	*3.5*

* Three $F_{2:3}$ families were tested

** Distance, in centimorgans, between marker and resistance gene.

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References

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